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<b>(21) International Application Number:</b> PCT/US89/01829 <b>(22) International Filing Date:</b> 1 May 1989 (01.05.89) <b>(30) Priority data:</b> 192,756 11 May 1988 (11.05.88) US <b>(71) Applicant:</b> EASTMAN KODAK COMPANY [US/US]; 343 State Street, Rochester, NY 14650 (US). <b>(72) Inventors:</b> BOWERS, Cyril, Yarling ; 484 Audubon Street, New Orleans, LA 70118 (US). MOMANY, Frank, Alden ; 1-1 Concord Green, Concord, MA 02154 (US). CODY, Wayne, Livingston ; Route 2, Box 65, Ringoes, NJ 08551 (US). HUBBS, John, Clark ; Route 10, Box 354A, King- sport, TN 37664 (US). FOSTER, Charles, Howard ; 1413 Dobyns Drive, Kingsport, TN 37664 (US).		<b>(74) Agent:</b> REITER, Stephen, E.; 343 State Street, Rochester, NY 14650 (US). <b>(81) Designated States:</b> AT (European patent), AU, BE (Euro- pean patent); CH (European patent), DE (European pa- tent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (Eu- ropean patent), SE (European patent).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> POLYPEPTIDE COMPOUNDS HAVING GROWTH HORMONE RELEASING ACTIVITY  <b>(57) Abstract</b>  Disclosed are novel polypeptide compounds which promote the release and elevation of growth hormone levels in the blood of animals. Also disclosed are methods of promoting the release and elevation of growth hormone levels in the blood of animals using the disclosed polypeptide compounds.		

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DescriptionPOLYPEPTIDE COMPOUNDS HAVING GROWTH HORMONE  
RELEASING ACTIVITY

This invention relates to novel polypeptide  
5 compounds which promote the release of growth hormone  
when administered to animals. In another aspect,  
this invention relates to methods for promoting the  
release and elevation of growth hormone levels in  
animals by administration of specified growth hormone  
10 releasing polypeptide compounds thereto.

Background of the Invention

It has been established in the scientific  
literature that the elevation of growth hormone (GH)  
levels in mammals upon administration of GH-releasing  
15 compounds can lead to enhanced body weight and to  
enhanced milk production if sufficiently elevated GH  
levels occur upon administration. Further, it is  
known that the elevation of growth hormone levels in  
mammals can be accomplished by application of known  
20 growth hormone releasing agents, such as the  
naturally occurring growth hormone releasing hormones.

The elevation of growth hormone levels in mammals  
can also be accomplished by application of growth  
hormone releasing peptides, some of which have been  
25 previously described, for example, by F. A. Momany in  
U.S. 4,223,019, U.S. 4,223,020, U.S. 4,223,021,  
U.S. 4,224,316, U.S. 4,226,857, U.S. 4,228,155,  
U.S. 4,228,156, U.S. 4,228,157, U.S. 4,228,158,  
U.S. 4,410,512 and U.S. 4,410,513.

30 Antibodies to the endogenous growth hormone  
release inhibitor, somatostatin (SRIF) have also been  
used to cause elevated GH levels. In this latter  
example, growth hormone levels are elevated by

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removing the endogenous GH-release inhibitor (SRIF) before it reaches the pituitary, where it inhibits the release of GH.

Each of these methods for promoting the elevation of growth hormone levels involve materials which are expensive to synthesize and/or isolate in sufficient purity for administration to a target animal. Short chain, low molecular weight, relatively simple polypeptides which are relatively inexpensive to prepare and have the ability to promote the release of growth hormone would be desirable because they should be readily and inexpensively prepared, easily modified chemically and/or physically, as well as readily purified and formulated; and they should have excellent transport properties.

#### Objects of the Invention

It is, therefore, an object of the present invention to provide novel growth hormone releasing compounds which are capable of promoting the release and elevation of growth hormone levels in the blood of animals.

It is another object of the present invention to provide methods for promoting the release and/or elevation of growth hormone levels in the blood of animals.

These and other objects of the present invention will become apparent from inspection of the following description and claims.

#### Statement of the Invention

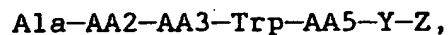
In accordance with the present invention, we have discovered novel polypeptide compounds which promote the release of growth hormone in animals. The preparation, characterization and administration of

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these novel growth hormone releasing compounds will now be described in greater detail.

#### Detailed Description of the Invention

The present invention is based on the discovery  
5 of short chain (i.e., six up to ten amino acid residues) polypeptides which promote the release and elevation of growth hormone levels in the blood of animals. The polypeptides contemplated to be within the scope of the present invention are defined by the  
10 following generic structure:



wherein

AA2 is selected from the group consisting of DPhe, DTrp, 5-fluoro-D or D/LTrp; 6-fluoro-D or  
15 D/LTrp (i.e., wherein the indole ring is fluorinated at the 5- or 6-position), (formyl)DTrp (i.e., DTrp which is formylated at the indole nitrogen), \*XTrp, wherein \*XTrp is selected from the group consisting of the N-monomethylated DTrp isomers (i.e.,  
20 (N<sup>α</sup>Me)DTrp and (indole NMe)DTrp), D<sup>α</sup>Nal and D<sup>β</sup>Nal;

AA3 is selected from the group consisting of Ala, Gly and Ser;

AA5 is selected from the group consisting of DPhe  
25 and (NMe)DPhe;

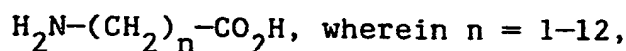
Y is selected from the group consisting of:

(a) AA7, wherein AA7 is selected from the group consisting of Arg, iLys, Lys and Orn; and

(b) -AA6-AA7, wherein AA6 is selected from  
30 the group consisting of all naturally occurring

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L-amino acids, dipeptides of the naturally occurring L-amino acids, e.g., Ala-Ala, and compounds of the formula:



5 and wherein AA7 is as defined above; and

Z represents the C terminal end group of said polypeptide or the C terminal amino acid(s) plus end group, wherein Z is selected from the group consisting of  $-\text{CONH}_2$ ,  $-\text{COOH}$ ,  $-\text{COOR}$ ,  $-\text{CONHR}$ ,  
 10  $-\text{CONR}_2$ ,  $-\text{CH}_2\text{OH}$  and  $-\text{CH}_2\text{OR}$ , wherein R is an alkyl group having 1-6 carbon atoms or an aromatic ring having up to 12 carbon atoms; and wherein Z is alternatively selected from the group consisting of  $-\text{Gly}-\text{Z}'$ ,  $-\text{Met}-\text{Z}'$ ,  $-\text{Lys}-\text{Z}'$ ,  $-\text{Cys}-\text{Z}'$ ,  $-\text{Gly}-\text{Tyr}-\text{Z}'$ , and  
 15  $-\text{Ala}-\text{Tyr}-\text{Z}'$ , wherein  $\text{Z}'$  is selected from the group consisting of  $-\text{CONH}_2$ ,  $-\text{CONHR}$ ,  $-\text{COOH}$ ,  $-\text{COOR}$ ,  $-\text{CONR}_2$ ,  $-\text{CH}_2\text{OH}$ , and  $-\text{CH}_2\text{OR}$ , wherein R is as defined above;

and organic or inorganic addition salts of any of  
 20 said polypeptides;

wherein the amino acid residue abbreviations used are in accordance with the standard peptide nomenclature:

25	Gly	= Glycine
	Tyr	= L-Tyrosine
	Ile	= L-Isoleucine
	Glu	= L-Glutamic Acid
	Thr	= L-Threonine
	Phe	= L-Phenylalanine

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	Ala	= L-Alanine
	Lys	= L-Lysine
	Asp	= L-Aspartic Acid
	Cys	= L-Cysteine
5	Arg	= L-Arginine
	Gln	= L-Glutamine
	Pro	= L-Proline
	Leu	= L-Leucine
	Met	= L-Methionine
10	Ser	= L-Serine
	Asn	= L-Asparagine
	His	= L-Histidine
	Trp	= L-Tryptophan
	Val	= L-Valine
15	DOPA	= 3,4-Dihydroxyphenylalanine
	Met(O)	= Methionine Sulfoxide
	Abu	= $\alpha$ -Aminobutyric Acid
	iLys	= N <sup>ε</sup> -Isopropyl-L-Lysine
	4-Abu	= 4-Aminobutyric Acid
20	Orn	= L-Ornithine
	D <sup>α</sup> Nal	= $\alpha$ -Naphthyl-D-Alanine
	D <sup>β</sup> Nal	= $\beta$ -Naphthyl-D-Alanine

All three letter amino acid abbreviations preceded by a "D" indicate the D-configuration of the amino acid residue, and abbreviations preceded by a "D/L" indicate a mixture of the D- and L-configurations of the designated amino acid. For purposes of this disclosure, glycine is considered to be included in the term "naturally occurring L-amino acids."

The flexibility associated with the choice of basic, neutral or acidic amino acid residues for amino acids AA2, AA3, AA5 and Y provides one with a great deal of control over the physiochemical

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properties of the desired peptide. Such flexibility provides important advantages for the formulation and delivery of the desired peptide to any given species. Additional flexibility can be imparted by  
5 the fact that the moieties R, Z and Z' can be varied as well, thereby providing added control over the physiochemical properties of the desired compound.

Preferred growth hormone releasing compounds employed in the practice of the present invention are  
10 selected from the group consisting of:

Ala-AA2-Ala-Trp-AA5-AA7-NH<sub>2</sub>,  
Ala-AA2-Ala-Trp-AA5-AA6-AA7-NH<sub>2</sub>, and

organic or inorganic addition salts of any of said polypeptides; where AA2, AA5, AA6 and AA7 are as  
15 defined above.

These compounds are preferred because of their ease of synthesis, proven efficacy at promoting an increase in serum growth hormone levels, and their consequent appeal for commercial scale production and  
20 utilization. In addition, these compounds may be advantageous in having physiochemical properties which are desirable for the efficient delivery of such polypeptide compounds to a variety of animal species. Because of the flexibility made possible by  
25 the various substitutions at numerous positions of the invention polypeptide compounds, a wide range of delivery vehicles can be employed, by selecting the polar, neutral or non-polar nature of the C-terminal and center portions of these polypeptide compounds so  
30 as to be compatible with the desired method of delivery.



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In a most preferred embodiment, the growth hormone releasing peptide employed in the practice of the present invention has the sequence:

Ala-AA2-Ala-Trp-AA5-AA7-NH<sub>2</sub>; or

5        organic or inorganic addition salts thereof, where AA2, AA5 and AA7 are as defined above. A particularly preferred member of this most preferred group of compounds has the sequence:

Ala-DTrp-Ala-Trp-DPhe-Lys-NH<sub>2</sub>, as well as  
10       organic or inorganic addition salts thereof.

These compounds are the presently most preferred because these shorter chain polypeptides are less expensive to synthesize, and these specific compounds have been shown to have a high level of potency at  
15       promoting the increase in serum growth hormone levels.

The compounds of this invention may be used to enhance blood GH levels in animals; enhance milk production in cows; enhance body growth in animals  
20       such as mammals (e.g., humans, sheep, bovines, and swine), as well as fish, fowl, other vertebrates and crustaceans; and increase wool and/or fur production in mammals. The amount of body growth is dependent upon the sex and age of the animal species, quantity  
25       and identity of the growth hormone releasing compound being administered, route of administration, and the like.

The novel polypeptide compounds of this invention can be synthesized according to the usual methods of solution and solid phase peptide chemistry, or by  
30       classical methods known in the art. The solid-phase

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synthesis is commenced from the C-terminal end of the peptide. A suitable starting material can be prepared, for instance, by attaching the required protected alpha-amino acid to a chloromethylated  
5 resin, a hydroxymethyl resin, a benzhydrylamine (BHA) resin, or a para-methyl-benzylhydramine (p-Me-BHA) resin. One such chloromethyl resin is sold under the tradename BIOBEADS SX-1 by Bio Rad Laboratories, Richmond, Calif. The preparation of the  
10 hydroxymethyl resin is described by Bodansky et al., Chem. Ind. (London) 38, 1597 (1966). The BHA resin has been described by Pietta and Marshall, Chem. Comm., 650 (1970) and is commercially available from Peninsula Laboratories, Inc., Belmont, California.

15 After the initial attachment, the alpha-amino protecting group can be removed by a choice of acidic reagents, including trifluoroacetic acid (TFA) or hydrochloric acid (HCl) solutions in organic solvents at room temperature. After removal of the  
20 alpha-amino protecting group, the remaining protected amino acids can be coupled stepwise in the desired order. Each protected amino acid can be generally reacted in about a 3-fold excess using an appropriate carboxyl group activator such as  
25 dicyclohexylcarbodiimide (DCC) or diisopropyl carbodiimide (DIC) in solution, for example, in methylene chloride ( $\text{CH}_2\text{Cl}_2$ ) or dimethylformamide (DMF) and mixtures thereof.

After the desired amino acid sequence has been  
30 completed, the desired peptide can be cleaved from the resin support by treatment with a reagent such as hydrogen fluoride (HF) which not only cleaves the peptide from the resin, but also cleaves most commonly used side-chain protecting groups. When a  
35 chloromethyl resin or hydroxymethyl resin is used, HF

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treatment results in the formation of the free peptide acid. When the BHA or p-Me-BHA resin is used, HF treatment results directly in free peptide amides.

5       The solid-phase procedure discussed above is well known in the art and has been described by Stewart and Young, Solid Phase Peptide Synthesis: Second Edn. (Pierce Chemical Co., Rockford, IL, 1984).

10       Some of the well known solution methods which can be employed to synthesize the peptide moieties of the instant invention are set forth in Bodansky et al., Peptide Synthesis, 2nd Edition, John Wiley & Sons, New York, N.Y. 1976.

15       In accordance with another embodiment of the present invention, a method is provided for promoting release and/or elevation of growth hormone levels in the blood of an animal. Said method comprises administering to an animal an effective dose of at least one of the above-described polypeptides.

20       The compounds of this invention can be administered by oral, parenteral (intramuscular (i.m.), intraperitoneal (i.p.), intravenous (i.v.) or subcutaneous (s.c.) injection), nasal, vaginal, rectal or sublingual routes of administration and can  
25 be formulated in dose forms appropriate for each route of administration.

30       Solid dose forms for oral administration include capsules, tablets, pills, powders and granules. In such solid dose forms, the active compound is mixed with at least one inert carrier such as sucrose, lactose, or starch. Such dose forms can also comprise, as is normal practice, additional substances other than inert diluents, e.g., lubricating agents such as magnesium stearate. In  
35 the case of capsules, tablets and pills, the dose

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forms may also comprise buffering agents. Tablets and pills can additionally be prepared with enteric coatings.

Liquid dose forms for oral administration include  
5 emulsions, solutions, suspensions, syrups, the elixirs containing inert diluents commonly used in the art, such as water. Besides, such inert diluents, compositions can also include adjuvants, such as wetting agents, emulsifying and suspending  
10 agents, and sweetening, flavoring, and perfuming agents.

Preparations according to this invention for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, or emulsions.  
15 Examples of non-aqueous solvents or vehicles are propylene glycol, polyethylene glycol, vegetable oils, such as olive oil and corn oil, gelatin, and injectable organic esters such as ethyl oleate. Such dose forms may also contain adjuvants such as  
20 preserving, wetting, emulsifying, and dispersing agents. They may be sterilized by, for example, filtration through a bacteria-retaining filter, by incorporating sterilizing agents into the compositions, by irradiating the compositions, or by  
25 heating the compositions. They can also be manufactured in a medium of sterile water, or some other sterile injectable medium immediately before use.

As suggested in our copending applications Serial  
30 No. 861,968 and S.N. 37,275, incorporated by reference herein, the novel compounds of the present invention are also believed to be useful when administered in combination with growth hormone releasing hormone (i.e., naturally occurring growth  
35 hormone releasing hormone, analogs and functional

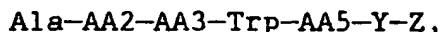
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equivalents thereof), as well as in combination with other compounds which promote the release of growth hormone. Such combinations represent an especially preferred means to administer the growth hormone releasing peptides of the present invention because the combination promotes the release of much more growth hormone than is predicted by the summation of the individual responses for each component of the combination, i.e., the combination provides a synergistic response relative to the individual component.

Combinations effective to cause the release and elevation of the level of growth hormone in the blood of an animal comprise an effective amount of polypeptides selected from at least two different groups of Group 1 polypeptides, Group 2 polypeptides or Group 3 polypeptides,

wherein Group 1 polypeptides are selected from any of the naturally occurring growth hormone releasing hormones and functional equivalents thereof, wherein said polypeptides act at the growth hormone releasing hormone receptor of mammals and other vertebrates, and crustaceans;

Group 2 polypeptides are selected from any of the polypeptides having the structure:



wherein AA2 is selected from the group consisting of DPhe, DTrp, 5-fluoro-D or D/LTrp; 6-fluoro-D or D/LTrp (i.e., wherein the indole ring is fluorinated at the 5- or 6-position), (formyl)DTrp (i.e., DTrp which is formylated at the indole nitrogen), \*XTrp, wherein \*XTrp is selected from the group consisting

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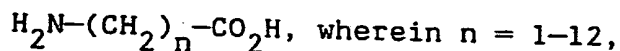
of the N-monomethylated DTrp isomers (i.e.,  
(N<sup>α</sup>Me)DTrp and (indole NMe)DTrp), D<sup>α</sup>Nal and  
D<sup>β</sup>Nal;

AA3 is selected from the group consisting of Ala,  
5 Gly and Ser;

AA5 is selected from the group consisting of DPhe  
and (NMe)DPhe;

Y is selected from the group consisting of:

- 10 (a) AA7, wherein AA7 is selected from the group  
consisting of Arg, iLys, Lys and Orn; and
- (b) -AA6-AA7, wherein AA6 is selected from the  
group consisting of all naturally occurring  
L-amino acids, dipeptides of the naturally  
15 occurring L-amino acids, e.g., Ala-Ala, and  
compounds of the formula:



and wherein AA7 is as defined above; and

Z represents the C terminal end group of said  
polypeptide or the C terminal amino acid(s) plus end  
20 group, wherein Z is selected from the group  
consisting of -CONH<sub>2</sub>, -COOH, -COOR, -CONHR,  
-CONR<sub>2</sub>, -CH<sub>2</sub>OH and -CH<sub>2</sub>OR, wherein R is an  
alkyl group having 1-6 carbon atoms or an aromatic  
ring having up to 12 carbon atoms; and wherein Z is  
25 alternatively selected from the group consisting of  
-Gly-Z', -Met-Z', -Lys-Z', -Cys-Z', -Gly-Tyr-Z', and  
-Ala-Tyr-Z', wherein Z' is selected from the group  
consisting of -CONH<sub>2</sub>, -COOH, -CONHR, -COOR,  
-CONR<sub>2</sub>, -CH<sub>2</sub>OH, and -CH<sub>2</sub>OR, wherein R is as  
30 defined above;

and organic or inorganic addition salts of any of  
said polypeptides;

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wherein the amino acid residue abbreviations used are in accordance with the standard peptide nomenclature:

	Gly	= Glycine
5	Tyr	= L-Tyrosine
	Ile	= L-Isoleucine
	Glu	= L-Glutamic Acid
	Thr	= L-Threonine
	Phe	= L-Phenylalanine
10	Ala	= L-Alanine
	Lys	= L-Lysine
	Asp	= L-Aspartic Acid
	Cys	= L-Cysteine
	Arg	= L-Arginine
15	Gln	= L-Glutamine
	Pro	= L-Proline
	Leu	= L-Leucine
	Met	= L-Methionine
	Ser	= L-Serine
20	Asn	= L-Asparagine
	His	= L-Histidine
	Trp	= L-Tryptophan
	Val	= L-Valine
	Abu	= $\alpha$ -Aminobutyric Acid
25	Sar	= Sarcosine
	Sar-ol	= Sarcosine Alcohol
	DOPA	= 3,4-Dihydroxyphenylalanine
	Gly-ol	= 2-Aminoethanol
	Hyp	= trans-4-Hydroxy-L-Proline
30	Met(O)	= Methionine sulfoxide
	Met(O)-ol	= Methionine sulfoxide alcohol
	Thz	= L-Thiazolidine-4- carboxylic Acid

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iLys	= N <sup>ε</sup> -Isopropyl-L-Lysine
4-Abu	= 4-Aminobutyric Acid
Orn	= L-Ornithine
D <sup>α</sup> Nal	= α-Naphthyl-D-Alanine
D <sup>β</sup> Nal	= β-Naphthyl-D-Alanine

5

All three letter amino acid abbreviations preceded by a "D" indicate the D-configuration of the amino acid residue; abbreviations preceded by a "D/L" indicate a mixture of the D- and L-configurations of the designated amino acids; and glycine is included in the scope of the term "naturally occurring L-amino acids";

Group 3 polypeptides are selected from any of the polypeptides having the structure:

- 15 Tyr-DArg-Phe-NH<sub>2</sub>  
 Tyr-DAla-Phe-NH<sub>2</sub>;  
 Tyr-DArg(NO<sub>2</sub>)-Phe-NH<sub>2</sub>;  
 Tyr-DMet(O)-Phe-NH<sub>2</sub>;  
 Tyr-DAla-Phe-Gly-NH<sub>2</sub>;  
 20 Tyr-DArg-Phe-Gly-NH<sub>2</sub>;  
 Tyr-DThr-Phe-Gly-NH<sub>2</sub>;  
 Phe-DArg-Phe-Gly-NH<sub>2</sub>;  
 Tyr-DArg-Phe-Sar;  
 Tyr-DAla-Gly-Phe-NH<sub>2</sub>;  
 25 Tyr-DArg-Gly-Trp-NH<sub>2</sub>;  
 Tyr-DArg(NO<sub>2</sub>)-Phe-Gly-NH<sub>2</sub>;  
 Tyr-DMet(O)-Phe-Gly-NH<sub>2</sub>;  
 (NMe)Tyr-DArg-Phe-Sar-NH<sub>2</sub>;  
 Tyr-DArg-Phe-Gly-ol;  
 30 Tyr-DArg-Gly-(NMe)Phe-NH<sub>2</sub>;  
 Tyr-DArg-Phe-Sar-ol  
 Tyr-DAla-Phe-Sar-ol  
 Tyr-DAla-Phe-Gly-Tyr-NH<sub>2</sub>;  
 Tyr-DAla-(NMe)Phe-Gly-Met(O)-ol;  
 35 Tyr-DArg-(NMe)Phe-Gly-Met(O)-ol;



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- Gly-Tyr-DArg-Phe-Gly-NH<sub>2</sub>;  
 Tyr-DThr-Gly-Phe-Thz-NH<sub>2</sub>;  
 Gly-Tyr-DAla-Phe-Gly-NH<sub>2</sub>;  
 Tyr-DAla-Phe-Gly-ol;  
 5 Tyr-DAla-Gly-(NMe)Phe-Gly-ol;  
 Tyr-DArg-Phe-Sar-NH<sub>2</sub>;  
 Tyr-DAla-Phe-Sar-NH<sub>2</sub>;  
 Tyr-DAla-Phe-Sar;  
 Tyr-DAla-Gly-(NMe)Phe-NH<sub>2</sub>;  
 10 Sar-Tyr-DArg-Phe-Sar-NH<sub>2</sub>;  
 Tyr-DCys-Phe-Gly-DCys-NH<sub>2</sub> (cyclic disulfide);  
 Tyr-DCys-Phe-Gly-DCys-NH<sub>2</sub> (free dithiol);  
 Tyr-DCys-Gly-Phe-DCys-NH<sub>2</sub> (cyclic disulfide);  
 Tyr-DCys-Gly-Phe-DCys-NH<sub>2</sub> (free dithiol);  
 15 Tyr-DAla-Phe-Gly-Tyr-Pro-Ser-NH<sub>2</sub>;  
 Tyr-DAla-Phe-Sar-Tyr-Pro-Ser-NH<sub>2</sub>;  
 Tyr-DAla-Phe-Sar-Phe-Pro-Ser-NH<sub>2</sub>;  
 Tyr-DAla-Phe-Gly-Tyr-Hyp-Ser-NH<sub>2</sub>;  
 Tyr-DAla-Phe-Sar-Tyr-Hyp-Ser-NH<sub>2</sub>;  
 20 Tyr-DAla-Phe-Sar-Phe-Hyp-Ser-NH<sub>2</sub>;  
 Tyr-DArg-Phe-Gly-Tyr-Hyp-Ser-NH<sub>2</sub>;  
 Tyr-DArg-Phe-Sar-Tyr-Pro-Ser-NH<sub>2</sub>;  
 Tyr-DArg-Phe-Sar-Tyr-Hyp-Ser-NH<sub>2</sub>;  
 Tyr-DArg-Phe-Gly-Tyr-Pro-Ser-NH<sub>2</sub>; and  
 25 organic or inorganic addition salts of any of  
 said polypeptides of Group 3;  
 wherein said combination is administered in a  
 ratio such that said combination is effective to  
 cause the synergistic release and elevation of growth  
 30 hormone in the blood of such animal.  
 Preferred Group 1 polypeptides are selected from  
 any of the polypeptides:  
 (a) having the following amino acid sequences in  
 Positions 1-44 (numbered from N terminus to  
 35 C terminus):

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- (#144) YADAIFTNSYRKVLGQLSARKLLQDIMSRRQGE-SNQERGARARL-X,  
 (#145) YADAIFTNSYRKVLGQLSARKLLQDIMSRRQGE-RNQEQQGARVRL-X,  
 5 (#146) YADAIFTNSYRKVLGQLSARKLLQDIMNRQQGE-RNQEQQAKVRL-X,  
 (#148) YADAIFTNSYRKILGQLSARKLLQDIMNRQQGE-RNQEQQAKVRL-X,  
 10 (#149) HADAIFTSSYRRILGQLYARKLLHEIMNRQQGE-RNQEQRSRFN-X; and functional equivalents thereof:

wherein the C-terminal amino acid has the following truncated general formula



wherein each R' independently represents the substituents of the particular amino acid residue, e.g.: hydrogen, alkyl, aryl, amino or acid substituents; X denotes the C terminal end group and is selected from  
 20 -CONH<sub>2</sub>, -COOH, -COOR, -CONRR, -CH<sub>2</sub>OH, and -CH<sub>2</sub>OR, where R is an alkyl group having 1 to 6 carbon atoms or an aromatic ring having up to 12 carbon atoms; and  
 25 wherein the amino acid residue abbreviations used are in accordance with the standard peptide nomenclature:

- G = Gly (Glycine),  
 Y = Tyr (L-Tyrosine),  
 30 I = Ile (L-Isoleucine),  
 E = Glu (L-Glutamic Acid),  
 T = Thr (L-Threonine),  
 F = Phe (L-Phenylalanine),

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- 5           A       = Ala (L-Alanine),  
             K       = Lys (L-Lysine),  
             D       = Asp (L-Aspartic Acid),  
             C       = Cys (L-Cysteine),  
             R       = Arg (L-Arginine),  
             Q       = Gln (L-Glutamine),  
             P       = Pro (L-Proline),  
             L       = Leu (L-Leucine),  
             M       = Met (L-Methionine),  
 10          S       = Ser (L-Serine),  
             N       = Asn (L-Asparagine),  
             H       = His (L-Histidine),  
             W       = Trp (L-Tryptophan), and  
             V       = Val (L-Valine);  
 15          Nle      = Norleucine  
             Sar      = Sarcosine  
             Sar-ol = Sarcosine Alcohol  
             Gly-ol = 2-Aminoethanol  
             Met(O) = Methionine Sulfoxide  
 20          (b) any one of said (a) polypeptides having the  
             following amino acid substitutions:
- Position 1 of (#144-#148) is DTyr or His;
- Position 1 of (#149) is Tyr or DHis;
- Position 2 of (#144-#149) is (NMe)DAla or  
 25          Aib or DAla;
- Position 3 of (#144-#149) is DAsp;
- Position 4 of (#144-#149) is DAla; and
- Position 1 + 2 of (#144-#149) is;

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DTyr<sup>1</sup> + DAla<sup>2</sup>, DTyr<sup>1</sup> + (NMe)DAla<sup>2</sup>,  
or DTyr<sup>1</sup> + Aib<sup>2</sup>;

- 5 (c) any one of said (a) or (b) polypeptides having a substitution of Nle for Met at Position 27;
- 10 (d) any one of said (a), (b) or (c) polypeptides in which the N-terminus -NH<sub>2</sub> is replaced by -NHCOR and wherein R is an alkyl group having 1 to 6 carbon atoms, or an aromatic ring having up to 12 carbon atoms;
- (e) fragments of any one of said (a), (b), (c) or (d) polypeptides which contain at least the amino acid residues of Positions 1-29;
- 15 (f) having the following specific amino acid sequences in Positions 1-29 (numbered from N terminus to C terminus):
- YADAIFTNSYRKVLQQLAARKLLQDIMSR-X,  
YADAIFTNSYRKVLQQLLARKLLQDIMSR-X,  
20 YSDAIFSNAIRKILQQLLARKLLQDIMQR-X,  
YADAIFSNAIRKILQQLLARKLLQDIMQR-X,  
YADAIFSSAIRRLLAQLASRLLQELLAR-X,  
YADAIFTNCRKVLQQLSARKLLQDIMSR-X  
(linear dithiol), and  
25 YADAIFTNCRKVLQQLSARKLLQDIMSR-X (cyclic disulfide);

wherein the C-terminal amino acid and X are as defined above; and modification of any one of these group (f) compounds in

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accordance with the modifications set forth in (b), (c) and (d) above; and

- (g) organic or inorganic addition salts of any of said (a), (b), (c), (d), (e) or (f) polypeptides of Group 1.

For further detail on the administration of combinations of growth hormone releasing peptides, those of skill in the art are referred to the above-cited applications.

- 10 The amount of polypeptide or combination of polypeptides of the present invention administered will vary depending on numerous factors, e.g., the particular animal treated, its age and sex, the desired therapeutic affect, the route of administration and which polypeptide or combination of polypeptides are employed. In all instances, however, a dose effective to promote release and elevation of growth hormone level in the blood of the recipient animal is used. Ordinarily, this dose level falls in the range of between about 0.1  $\mu$ g up to 10 mg of total polypeptide per kg of body weight. In general, the administration of combinations of growth hormone releasing peptides will allow for lower doses of the individual growth hormone releasing compounds to be employed relative to the dose levels required for individual growth hormone releasing compounds in order to obtain a similar response, due to the synergistic effect of the combination.

- Also included within the scope of the present invention are compositions comprising, as an active ingredient, the organic and inorganic addition salts of the above described polypeptides and combinations thereof; optionally, in association with a carrier, diluent, slow release matrix, or coating.

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The organic or inorganic addition salts of the growth hormone releasing compounds and combinations thereof contemplated to be within the scope of the present invention include salts of such organic  
5 moieties as acetate, trifluoroacetate, oxalate, valerate, oleate, laurate, benzoate, lactate, tosylate, citrate, maleate, fumarate, succinate, tartrate, naphthalate, and the like; and such  
inorganic moieties as Group I (i.e., alkali metal  
10 salts), Group II (i.e., alkaline earth metal salts), ammonium and protamine salts, zinc, iron, and the like with counterions such as the chloride, bromide, sulfate, phosphate and the like, as well as the organic moieties referred to above.

15 Pharmaceutically acceptable salts are preferred when administration to human subjects is contemplated. Such salts include the non-toxic alkali metal, alkaline earth metal and ammonium salts commonly used in the pharmaceutical industry  
20 including the sodium, potassium, lithium, calcium, magnesium, barium, ammonium and protamine salts which are prepared by methods well known in the art. The term also includes non-toxic acid addition salts which are generally prepared by reacting the  
25 compounds of this invention with a suitable organic or inorganic acid. Representative salts include the hydrochloride, hydrobromide, sulfate, bisulfate, acetate, oxalate, valerate, oleate, laurate, borate, benzoate, lactate, phosphate, tosylate, citrate,  
30 maleate, fumarate, succinate, tartrate, napsylate, and the like.

The invention will now be described in greater detail by reference to the following non-limiting examples.

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EXAMPLE 1 - Synthesis of the Growth Hormone Releasing Peptides

Paramethyl-benzhydrylamine hydrochloride (pMe-BHA·HCl) resin is placed in a reaction vessel  
 5 on a commercially available automated peptide synthesizer. The resin is substituted with free amine up to a loading of about 5 mmoles per gram. The compounds are prepared by coupling individual amino acids starting at the carboxy terminus of the  
 10 peptide sequence using an appropriate activating agent, such as N,N'-dicyclohexylcarbodiimide (DCC). The alpha amine functionalities of individual amino acids are protected, for example, as the t-butyloxycarbonyl derivative (t-Boc) and the reactive side chain  
 15 functionalities can be protected as outlined in Table 1.

Table 1

Side Chain Protecting Groups Suitable for  
 Solid Phase Peptide Synthesis

20	Arginine:	N <sup>B</sup> -Tosyl
	Aspartic Acid:	O-Benzyl
	Cysteine:	S-para-Methylbenzyl
	Glutamic Acid:	O-Benzyl
	Histidine:	N <sup>im</sup> -Tosyl
25	Lysine:	N <sup>E</sup> -2,4-Dichlorobenzyloxycarbonyl
	Methionine:	S-Sulfoxide
	Serine:	O-Benzyl
	Threonine:	O-Benzyl
	Tryptophan:	N <sup>in</sup> -Formyl
30	Tyrosine:	O-2,6-Dichlorobenzyl

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Prior to incorporation of the initial amino acid, the resin is agitated three times (about one minute each) with dichloromethane ( $\text{CH}_2\text{Cl}_2$ ; about 10 mL/gm of resin), neutralized with three agitations (about two minutes each) of N,N-diisopropylethylamine (DIEA) in dichloromethane (10:90; about 10 mL/gm of resin) and agitated three times (about one minute each) with dichloromethane (about 10 mL/gm of resin). The initial and each of the subsequent amino acids are coupled to the resin using a preformed symmetrical anhydride using about 3.0 times the total amount of the binding capacity of the resin of a suitably protected amino acid and about 1.5 times the total amount of the binding capacity of the resin of DCC in an appropriate amount of dichloromethane. For amino acids with a low dichloromethane solubility, N,N-dimethylformamide (DMF) is added to achieve a homogenous solution. Generally, the symmetrical anhydride is prepared up to 30 minutes prior to introduction into the reaction vessel at room temperature or below. The dicyclohexylurea that forms upon preparation of the symmetrical anhydride is removed via gravity filtration of the solution into the reaction vessel. Progress of the coupling of the amino acid to the resin is commonly monitored via a color test using a reagent such as ninhydrin (which reacts with primary and secondary amines). Upon complete coupling of the protected amino acid to the resin (>99%), the alpha amine protecting group is removed by treatment with acidic reagent(s). A commonly used reagent consists of a solution of trifluoroacetic acid (TFA), and anisole in dichloromethane (45:2:53).

After the desired amino acid sequence has been completed, the desired peptide can be cleaved from



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the resin support by treatment with a reagent such as hydrogen fluoride (HF) which not only cleaves the peptide from the resin, but also cleaves most commonly used side-chain protecting groups. When a  
5 chloromethyl resin or hydroxymethyl resin is used, HF treatment results in the formation of the free peptide acid. When the BHA or p-Me-BHA resin is used, HF treatment results directly in free peptide amides.

10 The complete procedure for incorporation of each individual amino acid residue onto the resin is outlined in Table 2.

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TABLE 2

Procedure for Incorporation of Individual Amino  
Acids onto a Resin

	<u>Reagent</u>	<u>Agitations</u>	<u>Time/Agitation</u>
5	1. Dichloromethane	3	1 min.
	2. TFA, Anisole, Dichloro- methane (45:2:53)	1	2 min.
	3. TFA, Anisole, Dichloro- methane (45:2:53)	1	20 min.
10	4. Dichloromethane	3	1 min.
	5. DIEA, Dichloromethane (10:90)	3	2 min.
	6. Dichloromethane	3	1 min.
15	7. Preformed symmetrical anhydride	1	15-120 min.*
	8. Dichloromethane	3	1 min.
	9. iso-Propanol	3	1 min.
	10. Dichloromethane	3	1 min.
20	11. Monitor progress of the coupling reaction**		
	12. Repeat Steps 1-12 for each individual amino acid		

\*Coupling time depends upon the individual amino acid.

25 \*\*The extent of coupling can be generally monitored by a  
color test. If the coupling is incomplete, the same  
amino acid can be recoupled by repeating Steps 7-11.  
If the coupling is complete the next amino acid can be  
coupled.

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EXAMPLE 2 - In Vivo GH Release in Rats

Immature female Sprague-Dawley rats were obtained from the Charles River Laboratories (Wilmington, MA). After arrival they were housed at 25° C with a  
5 14:10 hr light:dark cycle. Water and Purina rat chow were available ad libitum. Pups were kept with their mothers until 21 days of age.

Twenty-six day old rats, six rats per treatment group, were anesthetized interperitoneally with  
10 50 mg/kg of pentobarbital 20 minute prior to i.v. treatment with peptide. Normal saline with 0.1% gelatin was the vehicle for intravenous (i.v.) injections of the peptides. The anesthetized rats, weighing 55-65 grams, were injected i.v. with the  
15 quantity of growth hormone releasing compounds indicated in Table 3. Injection was made as a 0.1 mL solution into the jugular vein.

All animals were sacrificed by guillotine 10 minutes after the final test injection (see  
20 Table 3). Trunk blood for the determination of blood GH levels was collected following decapitation. After allowing the blood to clot, it was centrifuged and the serum was separated from the clot. Serum was kept frozen until the day of sampling for radio-  
25 immunoassay (RIA) determination of growth hormone levels according to the following procedure, as developed by the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases (NIADDK).

Reagents are generally added to the RIA analysis  
30 tubes at a single sitting, at refrigerator temperature (about 4°C) in the following sequence:

- (a) buffer,
- (b) "cold" (i.e., non-radioactive) standard or unknown serum sample to be analyzed,
- 35 (c) radio-iodinated growth hormone antigen, and
- (d) growth hormone antiserum.

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Reagent addition is generally carried out so that there is achieved a final RIA tube dilution of about 1:30,000 (antiserum to total liquid volume; vol:vol).

The mixed reagents are then typically incubated  
5 at room temperature (about 25°C) for about 24 hours prior to addition of a second antibody (e.g., goat or rabbit anti-monkey gamma globulin serum) which binds to and causes precipitation of the complexed growth hormone antiserum. Precipitated contents of the RIA  
10 tubes are then analyzed for the number of counts in a specified period of time in a gamma scintillation counter. A standard curve is prepared by plotting number of radioactive counts versus growth hormone (GH) level. GH levels of unknowns are then  
15 determined by reference to the standard curve.

Serum GH was measured by RIA with reagents provided by the National Hormone and Pituitary Program.

Serum levels in Table 3 are recorded in ng/mL in  
20 terms of the rat GH standard of 0.61 International Units/mg (IU/mg). Data is recorded as the mean +/- standard error of the mean (SEM). Statistical analysis was performed with Student's t-test. In Table 3 the results shown are the average of studies  
25 with six rats.

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Table 3

In Vivo GH Release (ng/mL) Promoted by Growth Hormone  
Releasing Compounds in Pentobarbital Anesthetized Rats  
(Animals Sacrificed 10 Minutes After Final Injection)

	5	Column A Growth Hormone Releasing Compounds	Total Dose ( $\mu$ g)	Control GH ng/mL	GH Released by Compound in Column A ng/mL
		His-DTrp-Ala-Trp-	0.3	208+47	660+128
10		DPhe-Lys-NH <sub>2</sub> *	1.0	208+47	1669+257
			3.0	208+47	3078+629
			0.3	142+43	533+ 77
			1.0	142+43	1656+213
			3.0	142+43	2653+378
			0.3**	108+17	764+ 62
			1.0**	108+17	2614+349
			3.0**	108+17	3215+547
15		His-Ala-DTrp-Ala-	0.3	151+16	261+ 32
		Trp-DPhe-Lys-NH <sub>2</sub> *	1.0	151+16	830+103
			3.0	151+16	2365+478
			3.0	111+25	2588+341
		Ala-DTrp-Ala-Trp-	0.3	208+47	265+ 96
20		DPhe-Lys-NH <sub>2</sub>	1.0	208+47	754+164
			3.0	208+47	1449+238
			0.3	142+43	303+ 41
			1.0	142+43	729+115
			3.0	142+43	1164+215
			10.0	142+43	2353+149
			30.0	142+43	2484+110
			1.0**	108+17	745+ 68
25			3.0**	108+17	1673+352
			10.0**	108+17	2388+269

\*Comparison Peptides

\*\*Doses so noted were administered to 29 day old  
30 female rats.

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In Table 3, a compound of the invention is shown to promote the release and elevation of growth hormone levels in the blood of rats to which such compounds have been administered. The surprising growth hormone releasing activity of this compound is potentially quite valuable since a short-chain, low molecular weight polypeptide with the relatively stable and inexpensive amino acid alanine at the amino-terminus should prove to be a low cost means to enhance growth hormone levels in animals.

EXAMPLE 3 - Administration of a Combination of GH-Releasing Compounds

The procedure of Example 2 was repeated, except the rats were not anesthetized nor were they pre-treated with pentobarbital, and a combination of peptides were administered to the rats. The compounds administered, the dose levels employed and results are set forth in Table 4.

TABLE 4

In Vivo Synergistic Effects in Unanesthetized Rats of Invention Compound with Group 1* Compound	
<u>Compound Administered; Dose (<math>\mu</math>g)*</u>	<u>GH Released, mg/mL</u>
Control	9 $\pm$ 0.9
Invention Compound, 10	75 $\pm$ 21
30	355 $\pm$ 141
Comparison Compound, 10	256 $\pm$ 66
Group 1 Compound, 10	144 $\pm$ 19

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TABLE 4 (Cont'd.)

In Vivo Synergistic Effects in Unanesthetized  
Rats of Invention Compound with Group 1\* Compound

	<u>Compound Administered; Dose (μg)*</u>	<u>GH Released, mg/mL</u>
5	Invention + Group 1, 10+10 30+10	2566+334 2020+214
	Comparison + Group 1, 10+10	2488+285

\*Group 1 compounds are described in detail in S.N.  
861,968 and 37,275, which have been incorporated  
10 by reference herein. All compounds employed for  
this example have the following sequences:

Invention Compound:

Ala-DTrp-Ala-Trp-DPhe-Lys-NH<sub>2</sub>

Comparison Compound:

15 His-DTrp-Ala-Trp-DPhe-Lys-NH<sub>2</sub>

Group 1 Compound:

Tyr-Ala-Asp-Ala-Ile-Phe-Thr-Asn-Ser-Tyr-Arg-Lys-  
Val-Leu-Gly-Gln-Leu-Ser-Ala-Arg-Lys-Leu-Leu-Gln-  
Asp-Ile-Nle-Ser-Arg-NH<sub>2</sub>

20 The results in Table 4 demonstrate that  
invention compound displays a similar synergistic  
response to that obtained with comparison compound  
(which has previously been shown to give a  
synergistic response) when administered in  
25 combination with an exemplary Group 1 compound.

The invention has been described in detail with  
particular reference to preferred embodiments there-  
of, but it will be understood that variations and  
modifications can be effected within the spirit and  
30 scope of the invention.

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CLAIMS

We Claim:

1. A polypeptide capable of promoting the release and elevation of growth hormone levels in the blood of a recipient animal, wherein said polypeptide is selected from the group consisting of polypeptides defined by the generic structure:

Ala-AA2-AA3-Trp-AA5-Y-Z,

wherein AA2 is selected from the group consisting of DPhe, DTrp, 5-fluoro-D or D/LTrp; 6-fluoro-D or D/LTrp (i.e., wherein the indole ring is fluorinated at the 5- or 6-position), (formyl)DTrp (i.e., DTrp which is formylated at the indole nitrogen), \*XTrp, wherein \*XTrp is selected from the group consisting of the N-monomethylated DTrp isomers (i.e., (N<sup>α</sup>Me)DTrp and (indole NMe)DTrp), D<sup>α</sup>Nal and D<sup>β</sup>Nal;

AA3 is selected from the group consisting of Ala, Gly and Ser;

AA5 is selected from the group consisting of DPhe and (NMe)DPhe;

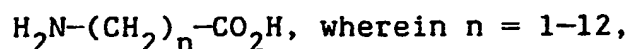
Y is selected from the group consisting of:

- (a) AA7, wherein AA7 is selected from the group consisting of Arg, iLys, Lys and Orn; and



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(b) -AA6-AA7, wherein AA6 is selected from the group consisting of all naturally occurring L-amino acids, dipeptides of the naturally occurring L-amino acids, e.g., Ala-Ala, and compounds of the formula:



and wherein AA7 is as defined above; and

Z represents the C terminal end group of said polypeptide or the C terminal amino acid(s) plus end group, wherein Z is selected from the group consisting of  $-\text{CONH}_2$ ,  $-\text{COOH}$ ,  $-\text{COOR}$ ,  $-\text{CONHR}$ ,  $-\text{CONR}_2$ ,  $-\text{CH}_2\text{OH}$  and  $-\text{CH}_2\text{OR}$ , wherein R is an alkyl group having 1-6 carbon atoms or an aromatic ring having up to 12 carbon atoms; and wherein Z is alternatively selected from the group consisting of  $-\text{Gly}-\text{Z}'$ ,  $-\text{Met}-\text{Z}'$ ,  $-\text{Lys}-\text{Z}'$ ,  $-\text{Cys}-\text{Z}'$ ,  $-\text{Gly}-\text{Tyr}-\text{Z}'$ , and  $-\text{Ala}-\text{Tyr}-\text{Z}'$ , wherein  $\text{Z}'$  is selected from the group consisting of  $-\text{CONH}_2$ ,  $-\text{COOH}$ ,  $-\text{CONHR}$ ,  $-\text{COOR}$ ,  $-\text{CONR}_2$ ,  $-\text{CH}_2\text{OH}$ , and  $-\text{CH}_2\text{OR}$ , wherein R is as defined above;

and organic or inorganic addition salts of any of said polypeptides;

wherein the amino acid residue abbreviations used are in accordance with the standard peptide nomenclature:

Gly	= Glycine
Tyr	= L-Tyrosine
Ile	= L-Isoleucine

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	Glu	= L-Glutamic Acid
	Thr	= L-Threonine
	Phe	= L-Phenylalanine
	Ala	= L-Alanine
5	Lys	= L-Lysine
	Asp	= L-Aspartic Acid
	Cys	= L-Cysteine
	Arg	= L-Arginine
	Gln	= L-Glutamine
10	Pro	= L-Proline
	Leu	= L-Leucine
	Met	= L-Methionine
	Ser	= L-Serine
	Asn	= L-Asparagine
15	His	= L-Histidine
	Trp	= L-Tryptophan
	Val	= L-Valine
	DOPA	= 3,4-Dihydroxyphenylalanine
	Met(O)	= Methionine Sulfoxide
20	Abu	= $\alpha$ -Aminobutyric Acid
	iLys	= N <sup>ε</sup> -Isopropyl-L-lysine
	4-Abu	= 4-Aminobutyric acid
	Orn	= L-Ornithine
	D <sup>α</sup> Nal	= $\alpha$ -naphthyl-D-alanine
25	D <sup>β</sup> Nal	= $\beta$ -naphthyl-D-alanine

All three letter amino acid abbreviations preceded by a "D" indicate the D-configuration of the amino acid residue; abbreviations preceded by a "D/L" indicate a mixture of the D- and L-configurations of the designated amino acids; and glycine is included in the scope of the term "naturally occurring L-amino acids".

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2. A polypeptide in accordance with Claim 1,  
wherein said polypeptide is selected from the  
group consisting of:

5                   Ala-AA2-Ala-Trp-AA5-AA7-NH<sub>2</sub>,  
                  Ala-AA2-Ala-Trp-AA5-AA6-AA7-NH<sub>2</sub>,

and

organic or inorganic addition salts of any of  
said polypeptides; wherein AA2, AA5, AA6 and AA7  
are as defined above.

- 10   3. A polypeptide in accordance with Claim 1 wherein  
said polypeptide has the sequence:

                  Ala-AA2-Ala-Trp-AA5-AA7-NH<sub>2</sub>, or  
                  organic or inorganic addition salts thereof,  
wherein AA2, AA5 and AA7 are as defined above.

- 15   4. A polypeptide in accordance with Claim 1 wherein  
said polypeptide has the sequence:

                  Ala-DTrp-Ala-Trp-DPhe-Lys-NH<sub>2</sub>; or

organic or inorganic addition salts thereof.

- 20   5. Method of promoting the release and elevation of  
blood growth hormone levels in animals by  
administering thereto an effective amount of at  
least one of the polypeptides set forth in  
Claim 1.

- 25   6. Method of promoting the release and elevation of  
blood growth hormone levels in animals by  
administering thereto an effective amount of at

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least one of the polypeptides set forth in Claim 2.

7. Method of promoting the release and elevation of blood growth hormone levels in animals by administering thereto an effective amount of at least one of the polypeptides set forth in Claim 3.
8. Method of promoting the release and elevation of blood growth hormone levels in animals by administering thereto an effective amount of at least one of the polypeptides set forth in Claim 4.
9. A combination effective to cause the release and elevation of the level of growth hormone in the blood of an animal, the combination comprising an effective amount of polypeptides selected from at least two different groups of Group 1 polypeptides, Group 2 polypeptides or Group 3 polypeptides,
- wherein Group 1 polypeptides are selected from any of the naturally occurring growth hormone releasing hormones and functional equivalents thereof, wherein said polypeptides act at the growth hormone releasing hormone receptor of mammals and other vertebrates, and crustaceans;
- Group 2 polypeptides are selected from any of the polypeptides having the structure:

Ala-AA2-AA3-Trp-AA5-Y-Z,

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wherein AA2 is selected from the group consisting of DPhe, DTrp, 5-fluoro-D or D/LTrp; 6-fluoro-D or D/LTrp (i.e., wherein the indole ring is fluorinated at the 5- or 6-position), (formyl)DTrp (i.e., DTrp which is formylated at the indole nitrogen), \*XTrp, wherein \*XTrp is selected from the group consisting of the N-monomethylated DTrp isomers (i.e., (N<sup>α</sup>Me)DTrp and (indole NMe)DTrp), D<sup>α</sup>Nal and D<sup>β</sup>Nal;

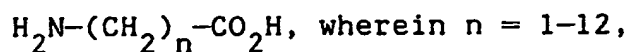
AA3 is selected from the group consisting of Ala, Gly and Ser;

AA5 is selected from the group consisting of DPhe and (NMe)DPhe;

Y is selected from the group consisting of:

(a) AA7, wherein AA7 is selected from the group consisting of Arg, iLys, Lys and Orn; and

(b) -AA6-AA7, wherein AA6 is selected from the group consisting of all naturally occurring L-amino acids, dipeptides of the naturally occurring L-amino acids, e.g., Ala-Ala, and compounds of the formula:



and wherein AA7 is as defined above; and

Z represents the C terminal end group of said polypeptide or the C terminal amino acid(s) plus end group, wherein Z is selected from the group

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consisting of  $-\text{CONH}_2$ ,  $-\text{COOH}$ ,  $-\text{COOR}$ ,  $-\text{CONHR}$ ,  
5  $-\text{CONR}_2$ ,  $-\text{CH}_2\text{OH}$  and  $-\text{CH}_2\text{OR}$ , wherein R is an  
alkyl group having 1-6 carbon atoms or an  
aromatic ring having up to 12 carbon atoms; and  
wherein Z is alternatively selected from the  
group consisting of  $-\text{Gly-Z}'$ ,  $-\text{Met-Z}'$ ,  $-\text{Lys-Z}'$ ,  
10  $-\text{Cys-Z}'$ ,  $-\text{Gly-Tyr-Z}'$ , and  $-\text{Ala-Tyr-Z}'$ , wherein  
Z' is selected from the group consisting of  
 $-\text{CONH}_2$ ,  $-\text{COOH}$ ,  $-\text{CONHR}$ ,  $-\text{COOR}$ ,  $-\text{CONR}_2$ ,  
15  $-\text{CH}_2\text{OH}$ , and  $-\text{CH}_2\text{OR}$ , wherein R is as defined  
above;

and organic or inorganic addition salts of any  
of said polypeptides;

15 wherein the amino acid residue abbreviations  
used are in accordance with the standard peptide  
nomenclature:

	Gly	= Glycine
	Tyr	= L-Tyrosine
	Ile	= L-Isoleucine
20	Glu	= L-Glutamic Acid
	Thr	= L-Threonine
	Phe	= L-Phenylalanine
	Ala	= L-Alanine
	Lys	= L-Lysine
25	Asp	= L-Aspartic Acid
	Cys	= L-Cysteine
	Arg	= L-Arginine
	Gln	= L-Glutamine
	Pro	= L-Proline
30	Leu	= L-Leucine
	Met	= L-Methionine
	Ser	= L-Serine

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	Asn	= L-Asparagine
	His	= L-Histidine
	Trp	= L-Tryptophan
	Val	= L-Valine
5	Abu	= $\alpha$ -Aminobutyric Acid
	Sar	= Sarcosine
	Sar-ol	= Sarcosine Alcohol
	DOPA	= 3,4-Dihydroxyphenylalanine
	Gly-ol	= 2-Aminoethanol
10	Hyp	= trans-4-Hydroxy-L-Proline
	Met(O)	= Methionine sulfoxide
	Met(O)-ol	= Methionine sulfoxide alcohol
	Thz	= L-Thiazolidine-4- carboxylic Acid
15	iLys	= N <sup>ε</sup> -Isopropyl-L-Lysine
	4-Abu	= 4-Aminobutyric Acid
	Orn	= L-Ornithine
	D <sup>α</sup> Nal	= $\alpha$ -Naphthyl-D-Alanine
20	D <sup>β</sup> Nal	= $\beta$ -Naphthyl-D-Alanine

All three letter amino acid abbreviations  
 preceded by a "D" indicate the D-configuration  
 of the amino acid residue; abbreviations  
 preceded by a "D/L" indicate a mixture of the D-  
 and L-configurations of the designated amino  
 acids; and glycine is included in the scope of  
 the term "naturally occurring L-amino acids".

Group 3 polypeptides are selected from any of  
 the polypeptides having the structure:

30 Tyr-DArg-Phe-NH<sub>2</sub>  
 Tyr-DAla-Phe-NH<sub>2</sub>;  
 Tyr-DArg(NO<sub>2</sub>)-Phe-NH<sub>2</sub>;  
 Tyr-DAla-(NMe)Phe-Gly-Met(O)-ol;  
 Tyr-DArg-(NMe)Phe-Gly-Met(O)-ol;

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- Tyr-DMet(O)-Phe-NH<sub>2</sub>;  
 Tyr-DAla-Phe-Gly-NH<sub>2</sub>;  
 Tyr-DArg-Phe-Gly-NH<sub>2</sub>;  
 Tyr-DThr-Phe-Gly-NH<sub>2</sub>;  
 5 Phe-DArg-Phe-Gly-NH<sub>2</sub>;  
 Tyr-DArg-Phe-Sar;  
 Tyr-DAla-Gly-Phe-NH<sub>2</sub>;  
 Tyr-DArg-Gly-Trp-NH<sub>2</sub>;  
 Tyr-DArg(NO<sub>2</sub>)-Phe-Gly-NH<sub>2</sub>;  
 10 Tyr-DMet(O)-Phe-Gly-NH<sub>2</sub>;  
 (NMe)Tyr-DArg-Phe-Sar-NH<sub>2</sub>;  
 Tyr-DArg-Phe-Gly-ol;  
 Tyr-DArg-Gly-(NMe)Phe-NH<sub>2</sub>;  
 Tyr-DArg-Phe-Sar-ol  
 15 Tyr-DAla-Phe-Sar-ol  
 Tyr-DAla-Phe-Gly-Tyr-NH<sub>2</sub>;  
 Gly-Tyr-DArg-Phe-Gly-NH<sub>2</sub>;  
 Tyr-DThr-Gly-Phe-Thz-NH<sub>2</sub>;  
 Gly-Tyr-DAla-Phe-Gly-NH<sub>2</sub>;  
 20 Tyr-DAla-Phe-Gly-ol;  
 Tyr-DAla-Gly-(NMe)Phe-Gly-ol;  
 Tyr-DArg-Phe-Sar-NH<sub>2</sub>;  
 Tyr-DAla-Phe-Sar-NH<sub>2</sub>;  
 Tyr-DAla-Phe-Sar;  
 25 Tyr-DAla-Gly-(NMe)Phe-NH<sub>2</sub>;  
 Sar-Tyr-DArg-Phe-Sar-NH<sub>2</sub>;  
 Tyr-DCys-Phe-Gly-DCys-NH<sub>2</sub> (cyclic disulfide);  
 Tyr-DCys-Phe-Gly-DCys-NH<sub>2</sub> (free dithiol);  
 Tyr-DCys-Gly-Phe-DCys-NH<sub>2</sub> (cyclic disulfide);  
 30 Tyr-DCys-Gly-Phe-DCys-NH<sub>2</sub> (free dithiol);  
 Tyr-DAla-Phe-Gly-Tyr-Pro-Ser-NH<sub>2</sub>;  
 Tyr-DAla-Phe-Sar-Tyr-Pro-Ser-NH<sub>2</sub>;  
 Tyr-DAla-Phe-Sar-Phe-Pro-Ser-NH<sub>2</sub>;  
 Tyr-DAla-Phe-Gly-Tyr-Hyp-Ser-NH<sub>2</sub>;  
 35 Tyr-DAla-Phe-Sar-Tyr-Hyp-Ser-NH<sub>2</sub>;



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Tyr-DAla-Phe-Sar-Phe-Hyp-Ser-NH<sub>2</sub>;  
 Tyr-DArg-Phe-Gly-Tyr-Hyp-Ser-NH<sub>2</sub>;  
 Tyr-DArg-Phe-Sar-Tyr-Pro-Ser-NH<sub>2</sub>;  
 Tyr-DArg-Phe-Sar-Tyr-Hyp-Ser-NH<sub>2</sub>;  
 5 Tyr-DArg-Phe-Gly-Tyr-Pro-Ser-NH<sub>2</sub>; and  
 organic or inorganic addition salts of any of  
 said polypeptides of Group 3;

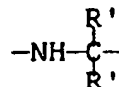
wherein said combination is administered in a  
 ratio such that said combination is effective to  
 10 cause the synergistic release and elevation of  
 growth hormone in the blood of such animal.

10. Combination of Claim 9 wherein said Group 1  
 polypeptides are selected from any of the  
 polypeptides:

15 (a) having the following amino acid sequences  
 in Positions 1-44 (numbered from N terminus  
 to C terminus):

(#144) YADAIFTNSYRKVLGQLSARKLLQDIMSRRQGE-  
 SNQERGARARL-X,  
 20 (#145) YADAIFTNSYRKVLGQLSARKLLQDIMSRRQGE-  
 RNQEQGARVRL-X,  
 (#146) YADAIFTNSYRKVLGQLSARKLLQDIMNRQGE-  
 RNQEQGAKVRL-X,  
 (#148) YADAIFTNSYRKILGQLSARKLLQDIMNRQGE-  
 25 RNQEQGAKVRL-X,  
 (#149) HADAIFTSSYRRILGQLYARKLLHEIMNRQGE-  
 RNQEQRSRFN-X; and functional  
 equivalents thereof:

wherein the C-terminal amino acid has the  
 30 following truncated general formula



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wherein each R' independently represents the substituents of the particular amino acid residue, e.g.: hydrogen, alkyl, aryl, amino or acid substituents; X denotes the C terminal end group and is selected from -CONH<sub>2</sub>, -COOH, -COOR, -CONRR, -CH<sub>2</sub>OH, and -CH<sub>2</sub>OR, where R is an alkyl group having 1 to 6 carbon atoms or an aromatic ring having up to 12 carbon atoms; and wherein the amino acid residue abbreviations used are in accordance with the standard peptide nomenclature:

	G	= Gly (Glycine),
	Y	= Tyr (L-Tyrosine),
15	I	= Ile (L-Isoleucine),
	E	= Glu (L-Glutamic Acid),
	T	= Thr (L-Threonine),
	F	= Phe (L-Phenylalanine),
	A	= Ala (L-Alanine),
20	K	= Lys (L-Lysine),
	D	= Asp (L-Aspartic Acid),
	C	= Cys (L-Cysteine),
	R	= Arg (L-Arginine),
	Q	= Gln (L-Glutamine),
25	P	= Pro (L-Proline),
	L	= Leu (L-Leucine),
	M	= Met (L-Methionine),
	S	= Ser (L-Serine),
	N	= Asn (L-Asparagine),
30	H	= His (L-Histidine),
	W	= Trp (L-Tryptophan), and
	V	= Val (L-Valine);
	Nle	= Norleucine
	Sar	= Sarcosine
35	Sar-ol	= Sarcosine Alcohol

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Gly-ol = 2-Aminoethanol

Met(O) = Methionine Sulfoxide

(b) any one of said (a) polypeptides having the following amino acid substitutions:

5           Position 1 of (#144-#148) is DTyr or His;

          Position 1 of (#149) is Tyr or DHis;

          Position 2 of (#144-#149) is (NMe)DAla or Aib or DAla;

          Position 3 of (#144-#149) is DAsp;

10           Position 4 of (#144-#149) is DAla; and

          Position 1 + 2 of (#144-#149) is;

          DTyr<sup>1</sup> + DAla<sup>2</sup>, DTyr<sup>1</sup> + (NMe)DAla<sup>2</sup>,  
          or DTyr<sup>1</sup> + Aib<sup>2</sup>;

15           (c) any one of said (a) or (b) polypeptides having a substitution of Nle for Met at Position 27;

20           (d) any one of said (a), (b) or (c) polypeptides in which the N-terminus -NH<sub>2</sub> is replaced by -NHCOR and wherein R is an alkyl group having 1 to 6 carbon atoms, or an aromatic ring having up to 12 carbon atoms;

25           (e) fragments of any one of said (a), (b), (c) or (d) polypeptides which contain at least the amino acid residues of Positions 1-29;

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- (f) having the following specific amino acid sequences in Positions 1-29 (numbered from N terminus to C terminus):

5 YADAIFTNSYRKVLQQLAARKLLQDIMSR-X,  
 YADAIFTNSYRKVLQQLLARKLLQDIMSR-X,  
 YSDAIFSNAYRKILQQLLARKLLQDIMQR-X,  
 YADAIFSNAYRKILQQLLARKLLQDIMQR-X,  
 YADAISSAYRRLLAQLASRRLQELLAR-X,  
 10 YADAIFTNCYRKVLCQLSARKLLQDIMSR-X  
 (linear dithiol), and  
 YADAIFTNCYRKVLCQLSARKLLQDIMSR-X (cyclic  
 disulfide);

15 wherein the C-terminal amino acid and X are  
 as defined above; and modification of any  
 one of these group (f) compounds in  
 accordance with the modifications set forth  
 in (b), (c) and (d) above; and

- 20 (g) organic or inorganic addition salts of any  
 of said (a), (b), (c), (d), (e) or (f)  
 polypeptides of Group 1.

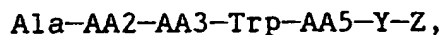
11. Combination of Claim 9 comprising a compound  
 from each of Group 1 polypeptides and Group 2  
 polypeptides.
12. Combination of Claim 9 comprising a compound  
 25 from each of Group 2 polypeptides and Group 3  
 polypeptides.
13. Combination of Claim 9 comprising a compound  
 from each of Group 1 polypeptides, Group 2  
 polypeptides and Group 3 polypeptides.

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14. Method of causing release and elevation of the level of growth hormone in the blood of an animal, comprising administering an effective dose of a combination comprising polypeptides selected from at least two different groups of Group 1 polypeptides, Group 2 polypeptides or Group 3 polypeptides,

wherein Group 1 polypeptides are selected from any of the naturally occurring growth hormone releasing hormones and functional equivalents thereof, wherein said polypeptides act at the growth hormone releasing hormone receptor of mammals and other vertebrates, and crustaceans;

Group 2 polypeptides are selected from any of the polypeptides having the structure:



wherein AA2 is selected from the group consisting of DPhe, DTrp, 5-fluoro-D or D/LTrp; 6-fluoro-D or D/LTrp (i.e., wherein the indole ring is fluorinated at the 5- or 6-position), (formyl)DTrp (i.e., DTrp which is formylated at the indole nitrogen), \*XTrp, wherein \*XTrp is selected from the group consisting of the N-monomethylated DTrp isomers (i.e., (N<sup>α</sup>Me)DTrp and (indole NMe)DTrp), D<sup>α</sup>Nal and D<sup>β</sup>Nal;

AA3 is selected from the group consisting of Ala, Gly and Ser;

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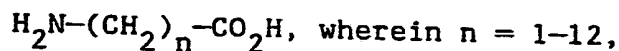
AA5 is selected from the group consisting of DPhe and (NMe)DPhe;

Y is selected from the group consisting of:

5 (a) AA7, wherein AA7 is selected from the group consisting of Arg, iLys, Lys and Orn; and

(b) -AA6-AA7, wherein AA6 is selected from the group consisting of all naturally occurring L-amino acids, dipeptides of the naturally occurring L-amino acids, e.g., Ala-Ala, and compounds of the formula:

10



and wherein AA7 is as defined above; and

15 Z represents the C terminal end group of said polypeptide or the C terminal amino acid(s) plus end group, wherein Z is selected from the group consisting of  $-\text{CONH}_2$ ,  $-\text{COOH}$ ,  $-\text{COOR}$ ,  $-\text{CONHR}$ ,  $-\text{CONR}_2$ ,  $-\text{CH}_2\text{OH}$  and  $-\text{CH}_2\text{OR}$ , wherein R is an alkyl group having 1-6 carbon atoms or an aromatic ring having up to 12 carbon atoms; and

20 wherein Z is alternatively selected from the group consisting of  $-\text{Gly}-\text{Z}'$ ,  $-\text{Met}-\text{Z}'$ ,  $-\text{Lys}-\text{Z}'$ ,  $-\text{Cys}-\text{Z}'$ ,  $-\text{Gly}-\text{Tyr}-\text{Z}'$ , and  $-\text{Ala}-\text{Tyr}-\text{Z}'$ , wherein  $\text{Z}'$  is selected from the group consisting of  $-\text{CONH}_2$ ,  $-\text{COOH}$ ,  $-\text{CONHR}$ ,  $-\text{COOR}$ ,  $-\text{CONR}_2$ ,  $-\text{CH}_2\text{OH}$ , and  $-\text{CH}_2\text{OR}$ , wherein R is as defined

25 above;

and organic or inorganic addition salts of any of said polypeptides;

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wherein the amino acid residue abbreviations used are in accordance with the standard peptide nomenclature:

	Gly	= Glycine
5	Tyr	= L-Tyrosine
	Ile	= L-Isoleucine
	Glu	= L-Glutamic Acid
	Thr	= L-Threonine
	Phe	= L-Phenylalanine
10	Ala	= L-Alanine
	Lys	= L-Lysine
	Asp	= L-Aspartic Acid
	Cys	= L-Cysteine
	Arg	= L-Arginine
15	Gln	= L-Glutamine
	Pro	= L-Proline
	Leu	= L-Leucine
	Met	= L-Methionine
	Ser	= L-Serine
20	Asn	= L-Asparagine
	His	= L-Histidine
	Trp	= L-Tryptophan
	Val	= L-Valine
	DOPA	= 3,4-Dihydroxyphenylalanine
25	Met(O)	= Methionine Sulfoxide
	Abu	= $\alpha$ -Aminobutyric Acid
	iLys	= N <sup>c</sup> -Isopropyl-L-Lysine
	4-Abu	= 4-Aminobutyric Acid
	Orn	= L-Ornithine
30	Sar	= Sarcosine
	Sar-ol	= Sarcosine Alcohol
	D <sup><math>\alpha</math></sup> Nal	= $\alpha$ -Naphthyl-D-Alanine
	D <sup><math>\beta</math></sup> Nal	= $\beta$ -Naphthyl-D-Alanine

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5 All three letter amino acid abbreviations preceded by a "D" indicate the D-configuration of the amino acid residue; abbreviations preceded by a "D/L" indicate a mixture of the D- and L-configurations of the designated amino acids; and glycine is included in the scope of the term "naturally occurring L-amino acids".

Group 3 polypeptides are selected from any of the polypeptides having the structure:

10 Tyr-DArg-Phe-NH<sub>2</sub>;  
 Tyr-DAla-Phe-NH<sub>2</sub>;  
 Tyr-DArg(NO<sub>2</sub>)-Phe-NH<sub>2</sub>;  
 Tyr-DMet(O)-Phe-NH<sub>2</sub>;  
 Tyr-DAla-Phe-Gly-NH<sub>2</sub>;  
 15 Tyr-DArg-Phe-Gly-NH<sub>2</sub>;  
 Tyr-DThr-Phe-Gly-NH<sub>2</sub>;  
 Phe-DArg-Phe-Gly-NH<sub>2</sub>;  
 Tyr-DArg-Phe-Sar;  
 Tyr-DAla-Gly-Phe-NH<sub>2</sub>;  
 20 Tyr-DArg-Gly-Trp-NH<sub>2</sub>;  
 Tyr-DArg(NO<sub>2</sub>)-Phe-Gly-NH<sub>2</sub>;  
 Tyr-DMet(O)-Phe-Gly-NH<sub>2</sub>;  
 (NMe)Tyr-DArg-Phe-Sar-NH<sub>2</sub>;  
 Tyr-DArg-Phe-Gly-ol;  
 25 Tyr-DArg-Gly-(NMe)Phe-NH<sub>2</sub>;  
 Tyr-DArg-Phe-Sar-ol  
 Tyr-DAla-Phe-Sar-ol  
 Tyr-DAla-Phe-Gly-Tyr-NH<sub>2</sub>;  
 Gly-Tyr-DArg-Phe-Gly-NH<sub>2</sub>;  
 30 Tyr-DThr-Gly-Phe-Thz-NH<sub>2</sub>;  
 Gly-Tyr-DAla-Phe-Gly-NH<sub>2</sub>;  
 Tyr-DAla-Phe-Gly-ol;  
 Tyr-DAla-Gly-(NMe)Phe-Gly-ol;  
 Tyr-DAla-(NMe)Phe-Gly-Met(O)-ol;  
 35 Tyr-DArg-(NMe)Phe-Gly-Met(O)-ol;



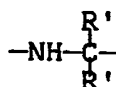
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- Tyr-DArg-Phe-Sar-NH<sub>2</sub>;  
 Tyr-DAla-Phe-Sar-NH<sub>2</sub>;  
 Tyr-DAla-Phe-Sar;  
 Tyr-DAla-Gly-(NMe)Phe-NH<sub>2</sub>;  
 5 Sar-Tyr-DArg-Phe-Sar-NH<sub>2</sub>;  
 Tyr-DCys-Phe-Gly-DCys-NH<sub>2</sub> (cyclic disulfide);  
 Tyr-DCys-Phe-Gly-DCys-NH<sub>2</sub> (free dithiol);  
 Tyr-DCys-Gly-Phe-DCys-NH<sub>2</sub> (cyclic disulfide);  
 Tyr-DCys-Gly-Phe-DCys-NH<sub>2</sub> (free dithiol);  
 10 Tyr-DAla-Phe-Gly-Tyr-Pro-Ser-NH<sub>2</sub>;  
 Tyr-DAla-Phe-Sar-Tyr-Pro-Ser-NH<sub>2</sub>;  
 Tyr-DAla-Phe-Sar-Phe-Pro-Ser-NH<sub>2</sub>;  
 Tyr-DAla-Phe-Gly-Tyr-Hyp-Ser-NH<sub>2</sub>;  
 Tyr-DAla-Phe-Sar-Tyr-Hyp-Ser-NH<sub>2</sub>;  
 15 Tyr-DAla-Phe-Sar-Phe-Hyp-Ser-NH<sub>2</sub>;  
 Tyr-DArg-Phe-Gly-Tyr-Hyp-Ser-NH<sub>2</sub>;  
 Tyr-DArg-Phe-Sar-Tyr-Pro-Ser-NH<sub>2</sub>;  
 Tyr-DArg-Phe-Sar-Tyr-Hyp-Ser-NH<sub>2</sub>;  
 Tyr-DArg-Phe-Gly-Tyr-Pro-Ser-NH<sub>2</sub>; and organic  
 20 or inorganic addition salts of any of said  
 polypeptides of Group 3.
15. Method of Claim 14 wherein said Group 1  
 polypeptides are selected from any of the  
 polypeptides:
- 25 (a) having the following amino acid sequences  
 in Positions 1-44 (numbered from N terminus  
 to C terminus):
- (#144) YADAIFTNSYRKVLGQLSARKLLQDIMSRQQGE-  
 SNQERGARARL-X,  
 30 (#145) YADAIFTNSYRKVLGQLSARKLLQDIMSRQQGE-  
 RNQEQGARVRL-X,  
 (#146) YADAIFTNSYRKVLGQLSARKLLQDIMNRQQGE-  
 RNQEQGAKVRL-X,

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- (#148) YADAIFTNSYRKILGQLSARKLLQDIMNRQQGE-  
RNQEQGAKVRL-X,  
(#149) HADAIFTSSYRRILGQLYARKLLHEIMNRQQGE-  
RNQEQRSRFN-X; and functional  
equivalents thereof:

wherein the C-terminal amino acid has the  
following truncated general formula



wherein each R' independently represents  
the substituents of the particular amino  
acid residue, e.g.: hydrogen, alkyl, aryl,  
amino or acid substituents; X denotes the C  
terminal end group and is selected from  
-CONH<sub>2</sub>, -COOH, -COOR, -CONRR, -CH<sub>2</sub>OH,  
and -CH<sub>2</sub>OR, where R is an alkyl group  
having 1 to 6 carbon atoms or an aromatic  
ring having up to 12 carbon atoms; and  
wherein the amino acid residue  
abbreviations used are in accordance with  
the standard peptide nomenclature:

G	= Gly (Glycine),
Y	= Tyr (L-Tyrosine),
I	= Ile (L-Isoleucine),
E	= Glu (L-Glutamic Acid),
T	= Thr (L-Threonine),
F	= Phe (L-Phenylalanine),
A	= Ala (L-Alanine),
K	= Lys (L-Lysine),
D	= Asp (L-Aspartic Acid),
C	= Cys (L-Cysteine),
R	= Arg (L-Arginine),

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Q = Gln (L-Glutamine),  
 P = Pro (L-Proline),  
 L = Leu (L-Leucine),  
 M = Met (L-Methionine),  
 5 S = Ser (L-Serine),  
 N = Asn (L-Asparagine),  
 H = His (L-Histidine),  
 W = Trp (L-Tryptophan), and  
 V = Val (L-Valine);  
 10 Aib =  $\alpha$ -Aminoisobutyric Acid  
 Nle = Norleucine  
 (NMe)DAIa = N-Methyl-D-Alanine

(b) any one of said (a) polypeptides having the following amino acid substitutions:

15 Position 1 of (#144-#148) is DTyr or His;

Position 1 of (#149) is Tyr or DHis;

Position 2 of (#144-#149) is (NMe)DAIa or Aib or DAIa;

Position 3 of (#144-#149) is DAsp;

20 Position 4 of (#144-#149) is DAIa; and

Position 1 + 2 of (#144-#149) is;

$\text{DTyr}^1 + \text{DAIa}^2$ ,  $\text{DTyr}^1 + (\text{NMe})\text{DAIa}^2$ ,  
 or  $\text{DTyr}^1 + \text{Aib}^2$ ;

25 (c) any one of said (a) or (b) polypeptides  
 having a substitution of Nle for Met at  
 Position 27;

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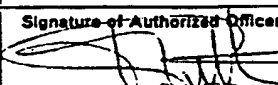
- (d) any one of said (a), (b) or (c) polypeptides in which the N-terminus  $\text{-NH}_2$  is replaced by  $\text{-NHCOR}$  and wherein R is an alkyl group having 1 to 6 carbon atoms, or an aromatic ring having up to 12 carbon atoms;
- (e) fragments of any one of said (a), (b), (c) or (d) polypeptides which contain at least the amino acid residues of Positions 1-29;
- (f) having the following specific amino acid sequences in Positions 1-29 (numbered from N terminus to C terminus):
- YADAIFTNSYRKVLQQLAARKLLQDIMSR-X,  
YADAIFTNSYRKVLQQLLARKLLQDIMSR-X,  
YSDAIFSNAYRKILQQLLARKLLQDIMQR-X,  
YADAIFSNAYRKILQQLLARKLLQDIMQR-X,  
YADAIFSSAYRRLLAQLASRRLQELLAR-X,  
YADAIFTNCYRKVLCQLSARKLLQDIMSR-X  
(linear dithiol), and  
YADAIFTNCYRKVLCQLSARKLLQDIMSR-X (cyclic disulfide);
- wherein the C-terminal amino acid and X are as defined above; and modification of any one of these group (f) compounds in accordance with the modifications set forth in (b), (c) and (d) above; and
- (g) organic or inorganic addition salts of any of said (a), (b), (c), (d), (e) or (f) polypeptides of Group 1.

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16. Method of Claim 14 wherein said combination  
comprises a compound from each of Group 1  
polypeptides and Group 2 polypeptides.
- 5 17. Method of Claim 14 wherein said combination  
comprises a compound from each of Group 2  
polypeptides and Group 3 polypeptides.
- 10 18. Method of Claim 14 wherein said combination  
comprises a compound from each of Group 1  
polypeptides, Group 2 polypeptides and Group 3  
polypeptides.

# INTERNATIONAL SEARCH REPORT

International Application No PCT/US 89/01829

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) <sup>6</sup>		
According to International Patent Classification (IPC) or to both National Classification and IPC		
IPC <sup>4</sup> : C 07 K 7/00, C 07 K 7/06, A 61 K 37/02		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>7</sup>		
Classification System	Classification Symbols	
IPC <sup>4</sup>	C 07 K 7/00, A 61 K 37/00	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>8</sup>		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT</b> <sup>9</sup>		
Category <sup>9</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
Y	EP, A, 0018072 (BECKMAN INSTRUMENTS) 29 October 1980 see pages 0-14, 18-31, 37-41 cited in the application --	1-4
P, Y	WO, A, 88/09780 (EASTMAN KODAK) 15 December 1988 see the whole document --	1-4
A	EP, A, 0083864 (BECKMAN INSTRUMENTS) 20 July 1983 see pages 0-7, 41-63, 117-123 cited in the application --	1, 2
A	WO, A, 87/06835 (EASTMAN KODAK) 19 November 1987 see pages 0-31, 74-105 -----	1, 2
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p><sup>10</sup> Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&amp;" document member of the same patent family</p> </div> </div>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search		Date of Mailing of this International Search Report
8th August 1989		01 SEP. 1989
International Searching Authority  EUROPEAN PATENT OFFICE		Signature of Authorized Officer  P.C.G. VAN DER PUTTEN

## FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND: incompletely searchable

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☒ Claim numbers .....\* because they relate to subject matter not required to be searched by this Authority, namely:

\* 5-8, 14, 15

See PCT-Rule 39.1(iv): Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods

2. ☒ Claim numbers 1, 9-13 because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Reason: Articles 5 and 6 PCT are violated to such an extent that a meaningful search cannot be carried out for the full scope of the claims 1, 9-13.

The search has therefore been limited to the compounds of claims 2-4

3. ☐ Claim numbers ..... because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING <sup>2</sup>

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

## Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

**ANNEX TO THE INTERNATIONAL SEARCH REPORT  
ON INTERNATIONAL PATENT APPLICATION NO.**

US 8901829

SA 28815

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 29/08/89. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0018072	29-10-80	US-A- 4223019	16-09-80
		US-A- 4228156	14-10-80
		US-A- 4228157	14-10-80
		US-A- 4228155	14-10-80
		US-A- 4224316	23-09-80
		US-A- 4226857	07-10-80
		US-A- 4228158	14-10-80
		US-A- 4223020	16-09-80
		US-A- 4223021	16-09-80
		AU-A- 5680180	02-10-80
		CA-A- 1175810	09-10-84
		JP-A- 61289098	19-12-86
		JP-A- 55133344	17-10-80
		JP-A- 59216860	06-12-84
WO-A- 8809780	15-12-88	US-A- 4839344	13-06-89
		AU-A- 2133688	04-01-89
EP-A- 0083864	20-07-83	US-A- 4410512	18-10-83
		US-A- 4410513	18-10-83
		US-A- 4411890	25-10-83
		AU-B- 549053	09-01-86
		CA-A- 1242435	27-09-88
		WO-A- 8302272	07-07-83
WO-A- 8706835	19-11-87	AU-A- 7433287	01-12-87
		EP-A- 0305401	08-03-89